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Freshwater Mussels As Indicators Of Healthy Ecosystems Based On An Integrative Ecosystem Life Cycle

Freshwater mussels (Unionoida) have seen significant drops in population numbers over the past decades. Of North America's 300 mussel species, almost 70% have become either extinct or considered endangered (Pandolfo *et al*. 2010). Reasons for these rapid decreases in populations can be attributed to many factors including pollution, climate change, water management, and habitat destruction. It is therefore important to understand what effects these factors may have on the mussels.

Freshwater mussels have a unique life cycle that consists of a parasitic larval stage (glochidium), a juvenile stage, and an adult stage. The glochidia must find a suitable host fish in order to complete the life cycle. Pollutions in waterways, however, prevent the metamorphosis from occurring, and affect mechanisms of survival for these larvae. Because glochidia respond negatively to pollution, less glochidia are surviving and becoming adults; therefore, ecosystem processes from the mussels are going to be negatively affected. Mussels contribute a number of functions on the ecosystem, including filtration of the water column, habitat construction, and nutrients recycling. Since mussels filter the water, nutrients that may be harmful in high concentrations to other organisms are taken out of the water column and transferred to into available nutrients. Additionally, mussels stimulate primary production by transferring nutrients to bacteria and algae. A loss of mussels, will therefore decrease primary production, and have a cascading effect among higher trophic levels. It is therefore important to start conservation efforts now to prevent a loss in ecosystem processes.

Freshwater mussels can be used to determine the health of freshwater ecosystems, because they respond negatively to increased toxins, their lifecycle requires various fish host, and their contribution to ecosystem functioning.

Cellular

As countries continue to develop and become more modernized, the use of chemicals such as fertilizers and insecticides increase. Many of these chemicals have also been found in significant concentrations in nearby waterways (Larson *et al.* 1999). These toxins are present in the sediment as well as free floating in the water column, and are taken up the food chain resulting in bioaccumulation in some instances, such as the heavy metal Cd or the infamous DDT compound (Frank and Gerstrmann 2007). Heavy metals are of special concern with freshwater mussels, due to their effects on growth, filtration efficiency, and enzyme activity (Naimo 1995). Also organic compounds, such as carbaryl, show a large effect on mussels. Both copper and carbaryl are found in many fertilizers and insecticides, and have been detected in significant concentrations in waterways (Conners and Black 2004). Copper, which is a heavy metal, stimulates the peroxidation of membrane lipids, and the peroxidation products like hydroperoxides and malondialdehydes are toxic to organisms due to functional impairments of cellular membranes (Eisler 1998). Furthermore, carbaryl, is a carbamate insecticide, that targets cholinesterase (Conners and Black 2004), which is a major enzyme responsible in the nervous system (Extoxnet 1993). In fact, a freshwater die-off due to inhibited cholinesterase has been documented, showing a need for cautious use of such products (Fleming *et al.* 1995). These mechanisms on the molecular level seem to be consistent throughout all stages of mussels; however, the responses to these toxics vary among the different stages.

Adult mussels seem to be more resilient to toxins than juveniles and glochidia; however, research is lacking in long-term effects on adults. Adults are seen to survive concentrations of copper more than ten times that of both juveniles and glochidia (Jacobson *et al.* 1997). However, these toxins may still affect the adult mussel that will decrease health conditions, resulting in long term mortality. High copper concentrations inhibit respiratory enzymes, which in turn increase ventilation rates and eventual suffocation of the mussel

(Alberta Environmental Protection 1996). High levels have also shown to decrease mussel filtration activity, which results in an increase in weight loss and finally starvation if conditions persist (Alberta Enviornment Protection 1996). Adult mussels are also more resilient to carbaryl. This seems to be linked to the fact that adult mussels have more mass; therefore, a higher concentration of the organic compound is needed to show any effects (Conners and Black 2004). Even though adults can withstand a higher concentration, mortality will eventually occur due to the strong effects on the nervous system. Juveniles are affected in the same way as adults; however, these effects are seen at lower concentrations, which again can be related to body mass (Conners and Black 2004). Adults do seem to have an advantage over both juveniles and glochidium, however, when concentrations are high enough mortality will occur.

Glochidium seem to present the most sensitivity to toxins in the water and sediments. Much of this sensitivity comes from the idea of size, however other mechanisms are affected from these toxins. Glochidia have considerably less mass than adults and most juveniles; therefore, lower concentrations place a threat (Conners and Black 2004). Copper places a major threat, because glochidia close their shells in response to the high concentrations (Alberta Enviornment Protection 1996). Once a glochidium initially closes its shell, it is not able to reopen it; therefore, mortality occurs from a failure to attach to a host (Arey 1920). Carbaryl, however, seems to affect glochidia in the same way as adults and juveniles, the only difference is in the concentration levels (Conners and Black 2004). Glochidia are a short life stage of the mussel, but high sensitivities to toxins greatly affect their survival and future success of mussel communities.

Population

Freshwater mussels have a unique life cycle that begins within the marsupial gill pouch of a female mussel (Arey 1920). Here the developing mussels progress into larvae called glochidia, which are eventually released into the water column where they attach to a host fish (Arey 1920). For successful metamorphosis and survival of the glochidium, it must first attach to a fish, typically on the gills or fins (Blažek and Gelnar 2006). Glochidia that do not attach to a host within days eventually die from starvation and lack of protection

(Dodd *et al.* 2006). The number of host fish varies considerably for each species of mussels, ranging from 1 to 37 (Watter 1994). This may be problematic if declines in these species of fish occur, due to mortality or evacuation of the area. After successful metamorphosis of the glochidium, the juvenile mussels are released and settle in the benthic sediments to further develop into reproductive adults (Arey 1920). This life cycle then provides a basis of study that can further help in the understanding and protection of these animals.

Glochidium attachment to a host fish becomes a crucial event, considering both metamorphosis and survival is dependent on it. Therefore, mussels have unique ways of attracting fish to increase attachment. Luring methods are one way some species of mussel increase host attachment. Modified mantle flaps on adult mussels mimic small fish, and the waving action of these flaps attract larger predatory fish (Haag and Warren 1999). As the fish approach the mantle flaps, the mussels are then able to efficiently infect the fish with the glochidia (Sietman *et al.* 2012). Another method that mussels use to improve host attachment is the release of large glochidial packages or superconglutinates. These packages resemble large invertebrates that fish feed on, and as the package is ingested, the glochidia are released to infect the gills of the fish (Haag and Warren 1999). Many species of glochidia also have hooks on their shells that help attach to the fins and gills of the host fish, however this is a species-specific trait (Taeubert *et al.* 2012). Once the glochidia are secured to the tissue, epidermal or branchial (gill) cells migrate and encapsulate the glochidium, forming a cyst, and once initiated usually takes 6 hours for completion (Arey 1920; Rogers-Lower and Dimock 2006). However, if a glochidium attaches to a non-suitable host, an abnormal cyst will form, resulting in a sloughing off and death of the glochidia (Fustish and Millemann1978). Glochidia attachment and encapsulation will therefore be an important aspect when looking at natural and resistant immunity of fish.

Glochidia can be defined as a parasitic stage of mussels, due to the fact that they can produce negative impacts on their host fish (Crane *et al.* 2011). These impacts are highly dependent on the density of glochidia present on a fish, and in extreme cases may cause mortality of the host (Howerth and Keller 2006). One study shows that parasitized fish use a "sit-and-wait" strategy when capturing prey (Crane *et al.* 2011). This strategy is most likely to conserve energy and reduce oxygen intake from damaged gills. However, even though parasitized fish exhibit energy saving strategies, they still show significant loss in body size (Crane *et al.* 2011). Parasitized fish also do not show a decrease in their foraging rate when threatened with predation (Crane *et al.* 2011). A decrease in body size, and an increase in foraging probably result from an increase in ventilation rate from damaged gills, and an increase in immunity response, which means more energy and hence more food is needed for survival (Dodd *et al.* 2005). Mussels, therefore, show a number of impacts on their host fish, therefore it is important for the survival of the fish to develop way of protection from infestations.

Immunity and resistance to glochidia by fish shows the nature of co-evolution between the two. Fish have developed ways of becoming resistant to glochidia both naturally and through multiple infections, and this resistance seems to occur through the formation of the cyst. This idea is evident in the delay of cyst formation in resistant fish. Constance Rogers showed that 48.0 % of cysts were fully developed in the normal 6 hour formation time span on naïve fish, compared to the 9.6 % of fully developed cysts in resistant fish (Rogers-Lowery and Dimock 2006). The slow cyst development in resistant fish allows the glochidia to be exposed to environmental impacts longer, which could be responsible for the poor host suitability. Along with natural immunity, it has been shown that multiple infections can lead to an acquired immunity. Constance Rogers showed a 45% and greater metamorphosis rate in the first two infections of a host fish, to less than 26% in following infections indicating an obvious source of acquired immunity (Rogers and Dimock 2003). A significant increase in shedding of dead glochidia has also been found in previously infected fish, possibly indicating a cellular response that was absent in the initial infections, which increased the death of the glochidia (Rogers and Dimock 2003). The resistance to the parasites also seems to remain for at least 12 months after priming (Dodd *et al.* 2006). Fish immunity to glochidia is an important aspect that will need further investigation and research on with conservation efforts and glochidia survival.

Environment

All organisms play a key role in their function on an ecosystem, and this function is a result of an expression of certain functional traits. Mussels in particular, have a major role in the function of the environment, through filtration of the water, nutrients cycle, and creation of diverse organismal habitats. In fact, rates of ecological processes by mussels show a linear relationship with the biomass of mussels (Vaughn *et al.* 2004). This shows that mussel's abundance and species richness has a large impact on ecosystem functions. Many of the effects that mussels provide to an ecosystem comes directly from their feeding behavior. Mussels are typically classified as epibenthic or half-buried in the sediment, with a foot that attaches them down into the streambed and a siphon that extends up into the water column (Vaughn *et al.* 2008). Since the mussels are half-buried in the sediment, they have multiple feeding opportunities. Mussels are able to use their foot to access food from the sediment which is classified as petal feeding, or they are can bend their inhalant siphons down into the sediment and vacuum up food sources which is classified as deposit feeding (Vaughn *et al.* 2008). Mussels also feed from the water column; however, the amount of material that mussels can filter out of the water column primarily depends on the volume and flow of the water source. Typically, streams and rivers have low flow and volume during mid summers months, which allows mussels to filter all of the passing water if populations are dense enough; however, in spring and winter months, water volume and flow exceeds the processing capacity of the mussels, leaving most of the water unfiltered (Vaughn *et al.* 2004). The maximum filtration rate of mussels has been observed at 1 L/h for a typical 61-mm-long mussel (Vaughn *et al.* 2008). This illustrates a need for massive populations of mussels in order to sufficiently filter a river or lake. It is important that these large aggregations (mussel beds) are rich in biodiversity, because mussels are thermo-conformers that adjust their metabolism rates based on surrounding temperatures (Spooner and Vaughn 2012). Different species of mussels tend to favor different temperatures in which they end up either filtering or excreting nutrients. Thermally tolerant species tend to perform higher ecosystem processes at higher temperatures, where as thermally sensitive species show a variety of responses, such as increase or decrease in filtration rate and nutrient excretion rates (Spooner and Vaughn 2012). Both species, however, are found co-existing together, and alternate in their dominance at different times of the year (Spooner and Vaughn 2012). It is important for an ecosystem to be biodiverse, due to this idea of alternating dominance. While some species may be active in filtration due to a tolerant environment, other non-tolerant species are excreting nutrients that will benefit other organisms.

The filtration process of mussels produces a nutrients cycle that allows other organisms to access these nutrients, which in turn creates a food web. Mussels link the benthic zone (sediment) and the pelagic zones (water column) through filtration and excretion of suspended matter to and from the water column (Vaughn 2010). Through the process of filtration, mussels can take nutrients that may be unavailable to other organism due to a lack of processing ability, and transfer it, so that they may become available to other organisms. Digestive enzyme examination has shown that the mussels can feed on nutrient-poor food sources, because they have proper enzymes for digestion (Christian *et al.* 2004). Mussels are then able to provide these nutrients to other organisms in the form of NH_3 and P, biodeposition of feces, and also by the release of nutrients via decay from dead mussels (Vaughn and Hakenkamp 2001). Primary production has been shown to increase significantly on surrounding sediment of the mussels, as well as on the shells themselves due to the excretion of these nutrients from mussels (Vaughn *et al.* 2008). Secondary production will therefore increase, due to an increase in primary production. This nutrients cycle that the mussels create, ultimately establishes a food web that will affect many organisms.

With the availably of nutrients to other organisms, as well as protection via shells, mussels establish habitats that are home to a number or organisms, such as bacteria, algae, and invertebrates. Mussels consume primary producers, thereby placing a direct impact on them; however, mussels also provide nutrients to primary producers, thereby placing an indirect impact on them (Vaughn *et al.* 2007). Algal growth is stimulated from the available nutrients, and because of the close proximity of these nutrients, the algae readily colonizes the shells of mussels (Vaughn *et al.* 2008). Since algae growth is increased on and around the mussels, grazing invertebrates also congregate around the mussel beds and benefit from the available food and shelter provided by the mussels. In fact, macroinvertebrates are seen at significantly higher densities inside mussel beds than outside of them (Spooner and Vaughn

2006). Mussels can also be seen as ecosystem engineers for habitat creation. Ecosystem engineers are species that modify the environment and greatly affect other species and ecosystem processes (Jones *et al.* 1994). Mussels can change the environment in the way sediments are transported during periods of high water flow. The size, shape, and shell morphology of mussels all play a role in sediment erosion and transport. Smooth shells create less turbulence, therefore less sediment transport occurs; however, rough shells create more turbulence, therefore, more sediment transport occurs (Allen and Vaughn 2011). Also the position of the shells affects sediment transport and erosion. Shell faces that are directed against the current create more turbulence, where as shell faces directed with the current create less turbulence (Allen and Vaughn 2011). Finally, mussels contribute to the stabilization and destabilization of the streambeds. Dense mussel beds compact and stabilize streambeds during floods and high water flow periods (Vaughn *et al.* 2008). Alternatively, burrowing activities of mussels create a turbulence of sediments, which in turn increases nutrients into the water column (Vaughn *et al.* 2008). It is thought that the turbulence of sediments stimulates microbial metabolism in these sediments, which creates more food availability for invertebrates and mussels, further improving the habitat (Vaughn *et al.* 2008). With that transfer of nutrients to other organisms, protection for organisms via shells, and a stability of the streambed, mussels generate a diverse habitat that is filled with a number of organisms.

Summary

Freshwater mussels are key indicators of healthy ecosystems, because of the negative effects to toxins like copper and carbaryl on glochidia and adult mussels, the life cycle and significance of host encystment on glochidia, and the ecosystem functions such as filtration, nutrients cycle and habitat creation. Toxins have clearly shown to negatively affect mussels. Though mortality due to high levels of copper has not been seen in adults in short-term studies, long-term results from decreased filtering and increased respiration are thought to end in death. Glochidia, however, have showed significant effects to toxins. High concentrations of copper resulted in a closure of the shell with the inability to reopen. The closure of the shell prevents the glochidia from attaching to a host fish. It is therefore important for the survival of the glochidia to become encysted on a host fish as quick as possible (Jacobson *et al*. 1997). The encapsulation of the glochidia from the host epithelial tissue seems to create a barrier from the environment and helps in the protection of the glochidia (Jacobson *et al*. 1997). If glochidia are not able to attach to a fish due to the effects of toxins, then this life stage of the mussel becomes threatened. Without the cyst formation of glochidia, metamorphosis cannot occur, resulting in an uncompleted life cycle. When the life cycle of the mussel is not completed, less adult biomass occurs in freshwater environments, resulting in a decrease of ecosystem processes. There will be a degradation of streambed stability, habitat creation, and filtration in waterways. As streambeds erode from the lack of stabilization from mussels, nutrients will mixed into the water column; however, with the lack of filtration to convert those nutrients into available nutrients to other organism, a concentration buildup will occur. When concentrations become too high, toxic level will occur causing a further decline in the health of the ecosystem. It is therefore imperative to understand all interactions and roles that mussels have in the ecosystem to prevent such a catastrophic effect to freshwater environments.

Research on these bivalves is numerous due to the strong influence they have in freshwater ecosystems. However, research is lacking in some fields. There are not many sources or studies on the long-term effects of toxins on adult mussels. Mussels can live more the 100 years, so long-term effects will occur (Galbraith *et al.* 2010). This may require expensive long-term studies, but will be necessary in examining the future of these declining species. Research also seems to be missing on the effects that toxins within host fish tissue play on the glochidia. During time of encapsulation, glochidia obtain nutrients from the tissue of the host fish (Rogers-Lowery and Dimock 2006). Any toxins in the tissue will be transmitted to the feeding glochidia, so it is expected that a negative effect will occur on the growing mussels. Understanding these effects will help contribute to the understanding of the decline in these once numerous species of bivalves, and may help with conservation efforts.

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